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Occurrence of Toxigenic *Aspergillus versicolor* Isolates and Sterigmatocystin in Carpet Dust from Damp Indoor Environments

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Over the past decade, there has been growing concern regarding the role of toxigenic fungi in damp indoor environments; however, there is still a lack of field investigations on exposure to mycotoxins. The goal of our pilot study was to quantify the proportion of toxigenic *Aspergillus versicolor* isolates in native carpet dust from damp dwellings with mold problems and to determine whether sterigmatocystin can be detected in this matrix. Carpet dust samples ($n = 11$) contained from $<2.5 \times 10^1$ to 3.6×10^5 (median, 3.1×10^4) *A. versicolor* CFU/g of dust, and the median proportion of *A. versicolor* from total culturable fungi was 18%. Based on thin-layer chromatography detection of sterigmatocystin, 49 of 50 *A. versicolor* isolates (98%) were found to be toxigenic in vitro. By using high-performance liquid chromatography–electrospray ionization tandem mass spectrometry, sterigmatocystin could be detected in low concentrations (2 to 4 ng/g of dust) in 2 of 11 native carpet dust samples. From this preliminary study, we conclude that most strains of *A. versicolor* isolated from carpet dust are able to produce sterigmatocystin in vitro and that sterigmatocystin may occasionally occur in carpet dust from damp indoor environments. Further research and systematic field investigation are needed to confirm our results and to provide an understanding of the health implications of mycotoxins in indoor environments.

Over the past decade, there has been growing concern regarding the role of toxigenic fungi in damp indoor environments (6, 18, 37, 43). Several epidemiological studies indicate that children and adults living in damp indoor environments are more likely to suffer from respiratory as well as general symptoms (e.g., tiredness, headaches, nausea, and vomiting), and exposure to fungi has been suggested as being an important contributory factor (1, 3, 4, 7, 32, 34, 35). The underlying mechanisms, however, are not well known (17, 33). Case reports and studies of agricultural workers indicate that certain health effects occur as a result of inhalation of molds, which are due at least in part to mycotoxins (6, 8, 9). To date, however, relatively few studies have examined exposure to mycotoxins in indoor environments. The current scientific literature on this topic focuses mainly on trichothecene mycotoxins produced by *Stachybotrys chartarum* (10, 13, 16). In addition, *Aspergillus versicolor* is presumed to be of concern, because it belongs to the most frequently occurring species found in damp indoor environments (19, 27) and is known to be the major producer of the hepatotoxic and carcinogenic mycotoxin sterigmatocystin (2, 5). In a study of water-damaged building materials, analyses of wallpaper and fiberglass wallpaper naturally contaminated with *A. versicolor* revealed sterigmatocystin and 5-methoxysterigmatocystin (30). Tuomi and coworkers found sterigmatocystin in 24% of bulk samples of moldy interior finishes from Finnish buildings with moisture problems, ranging from 0.2 to 1,000 ng per g (fresh weight) of sample (42).

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Sterigmatocystin is closely related to aflatoxin mycotoxins and is a precursor of aflatoxin biosynthesis (2); however, the acute and chronic toxicities of sterigmatocystin are considerably lower (39). There are no case reports or epidemiological data referring directly to human toxicology. Sterigmatocystin is carcinogenic in mice (pulmonary adenocarcinomas) and rats (hepatocellular carcinomas at milligram doses of sterigmatocystin per animal per day for 1 year) following oral administration (21) and is classified as an International Agency for Research on Cancer class 2B carcinogen (i.e., as possibly carcinogenic to humans) (22). The toxicity of sterigmatocystin refers primarily to liver and kidney. Oral dosing of monkeys with 20 mg/kg of body weight once each fortnight for 4 to 6 months resulted in chronic hepatitis; after a 12-month exposure, hyperplastic liver nodules were observed (21). In this paper, we present the results of a pilot study, the goal of which was to determine whether toxigenic *A. versicolor* isolates and sterigmatocystin can be detected in native carpet dust from damp dwellings with mold problems and to provide a preliminary assessment of the possible exposure of toddlers and preschool children to sterigmatocystin.

MATERIALS AND METHODS

Sampling locations. For environmental sampling, eight dwellings located in Hamburg, northern Germany, with visible mold growth and/or signs of indoor dampness were included in the pilot study (Table 1). The inhabitants had contacted the local health administration with questions regarding a possible relationship between their health problems and the presence of mold in their dwelling. The inhabitants were informed as to the purpose of the study and were instructed on how to collect the samples.

Environmental dust sampling. Carpet dust samples (samples 1 and 8, conventional carpets; samples 2 to 7, wall-to-wall carpets) were collected by the inhabitants with a conventional household vacuum cleaner with a power level of at

TABLE 1. Characterization of sampling locations by dates of construction and sampling, floor level, room type, and moisture and mold status as characterized by the inhabitants

Dwelling no.	Date of construction	Date of sampling	Floor	Room type (sample letter)	Presence or absence of[a]:		
					Visible mold	Moldy odors	Moisture damage
1	1900	July 1999	3rd	Kitchen	−	−	+
2	1963	July 1999	8th	Living room/bedroom	+	+	+
3	1955	November 1999	Below roof	Bedroom (a), Living room (b)	+	−	+
4	1977	March 2000	Ground level	Bedroom/children's room	+	(+)	+
5	1977	March 2000	3rd	Bedroom/children's room/kitchen	−	−	+
6	1977	April 2000	4th	Children's room (a), bedroom (b), office (c)	+	(+)	+
7	1954	May 2000	4th	Living room	+	−	+
8	1967	May 2000	1st	Bathroom	+	−	+

[a] +, present; (+), questionable; −, absent.

least 800 W and a new dust bag. After sampling for 2 min/m^2, the dust bags were closed with plastic tape and sent to the laboratory by mail (at ambient temperature). The area of the carpet surface sample varied from approximately 2 to 28 m^2 (median, 6.4 m^2). Most samples were collected from November 1999 to May 2000 (Table 1). The inhabitants were advised to complete a short sampling report, including parameters for moisture and visible mold growth.

Mycological investigations. Immediately after receipt of the samples, mycological investigations were carried out as described elsewhere (43). In brief, the dust samples were sieved (355-μm pore diameter) and weighed, and an aliquot of 40 mg was diluted in sterile water with 0.01% Tween 80. The remaining quantity of the sieved dust samples was stored at −20°C until further preparation for high-performance liquid chromatography (HPLC). After serial dilution, the dust samples were inoculated onto duplicate dichloran–18% glycerol (DG18) agar plates (Oxoid, Wesel, Germany) and incubated at 25°C. After 7 days, the plates were counted, and colonies suggestive of *A. versicolor* were isolated on Czapek Dox agar (Merck, Darmstadt, Germany). Species identification was based on characteristic morphology (36). The results were expressed in terms of CFU per gram of dust.

TLC. One-direction thin-layer chromatography (TLC) was carried out as described elsewhere (11, 14). In brief, selected isolates were inoculated onto Czapek Dox agar. These subcultures were incubated at 25°C for 14 days. Using a scalpel, we removed an agar plug (size, 4 by 4 mm) from the mold culture and wet the mycelium on the plug with a drop of methanol-chloroform (1:2 [vol/vol]). This treatment extracted the intracellular mycotoxins within seconds, after which they were transferred directly to a TLC plate (silica gel 60, item 5721-7; Merck, Darmstadt, Germany [activated for 2 h at 110°C]) by immediately placing the plug on the plate while the mycelium was still wet. After immediate removal of the plug, the plate was developed in toluene-ethyl acetate-formic acid (5:4:1 [vol/vol/vol]) and external standards (sterigmatocystin; Sigma Chemical Co., St. Louis, Mo.; and griseofulvin, Fluka BioChemica, Buchs, Switzerland). After this procedure, the plates were dried. Visualization was performed by spraying the TLC plate with a 20% (vol/vol) solution of anhydrous aluminum chloride in 96% ethanol and heating it for 5 min at 80°C. The spots were visualized under UV light at 366 nm. For detection, the spot color and the R_f value were analyzed.

HPLC-ESI-MS/MS. (i) Sample preparation. Samples were prepared as follows. One gram of dust (particle size, <355 μm) was weighed into a 10-ml glass vessel, and 5 ml of acetonitrile (HPLC grade; Merck) was added. The mixture was stirred by ultrasonic bath (Sonorex RK 102 H; Bandelin, Berlin, Germany) for 10 min, and the suspension was filtered through a glass-fiber filter (GF 6; Schleicher & Schuell, Dassel, Germany). For exhaustive extraction, the procedure was repeated with 3 ml of acetonitrile. The acetonitrile was completely removed from the filtrate under a nitrogen stream at room temperature, and the residue was resolved in 1 ml of methanol-water (50:50) (HPLC grade; Merck). The methanol-water solution was filtered again through a glass-fiber filter, and the filtrate was used for HPLC-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) detection. The level of recovery was 33% with dust samples (1.0 g) spiked at a level of 10 ng/g.

(ii) HPLC conditions. HPLC analysis was performed with a Series 1100 binary pump with an autosampler and column oven (Agilent Technologies, Waldbronn, Germany). Sterigmatocystin was separated from waste by isocratic elution on a 50-by-2.1-mm inside-diameter INERTSIL C_8 (5 μm) reversed-phase column (GL Sciences, Inc., Tokyo, Japan) maintained at 30°C. The mobile phase consisted of methanol with 8 mmol of aqueous ammonium acetate per liter (70:30) at a flow rate of 0.2 ml/min, providing a retention time for sterigmatocystin of approximately 2.3 min. The injection volume was 10 μl.

(iii) ESI-MS/MS parameters. Positive-ion ESI-MS/MS was performed with an API 2000 triple-stage quadrupole mass spectrometer with a TurboIon source (Applied Biosystems, Langen, Germany). At a pressure of 175 kPa, nitrogen was used as a curtain gas, and at a pressure of 280 kPa, nitrogen was used as a nebulizer gas. Nitrogen was also used as a drying gas at a pressure of 560 kPa and a temperature of 375°C. The interface had a temperature of 60°C. MS/MS parameters for the isolation of the precursor ion (proton adduct) and the product ions after fragmentation (multiple reaction monitoring [MRM] mode) were optimized in the continuous-flow mode, as shown in Table 2. The intensity of both major fragments was used for quantification purposes. The samples were analyzed together with a series of calibration standards of the blank (mobile phase): 1, 2, 4, 6, 8, and 10 ng/ml (sterigmatocystin standard lot 116H4079; Sigma Chemical Co., St. Louis, Mo.). The correlation coefficient, *r*, was 0.9998. Quantification was achieved by comparing the peak areas of both MRMs with the corresponding calibration plot of the standards (Fig. 1). The detection limit achieved by this method was 1 ng/g.

RESULTS

Mycological investigations. A total of 11 carpet dust samples from eight different households were analyzed (Table 3). The concentration of culturable fungi ranged from 1.0×10^3 to 3.2×10^6 CFU/g of dust (median, 1.4×10^5 CFU/g of dust). Ten of the 11 samples (91%) yielded culturable *A. versicolor* ranging from 0.8×10^2 to 3.6×10^5 CFU/g of dust (median, 3.1×10^4 CFU/g of dust). The proportion of culturable *A. versicolor* ranged from <0.1% to 80% (median, 18%).

TLC. A total of 50 *A. versicolor* isolates from the different carpet dust samples were tested for their ability to produce sterigmatocystin in vitro. Forty-nine of the 50 isolates (98%) tested were sterigmatocystin positive.

HPLC-ESI-MS/MS. Only 2 of 11 carpet dust samples (18%) exceeded the detection limit (1.0 ng of sterigmatocystin/g of dust), showing concentrations between 2 and 4 ng of sterig-

TABLE 2. MS/MS parameters for positive-ion ESI-MS detection of sterigmatocystin

Parameter	Value
MRM (*m/z*)	325.0→310.0, 325.0→281.0
Ion spray voltage (V)	5,500
Declustering potential (V)	102
Focusing potential (V)	380
Entrance potential (V)	−10
Collision cell entrance potential (V)	16
Collision energy (V)	35, 49
Collision cell exit potential (V)	5.5, 4.0
CAD[a] gas thickness (mol/cm^2)	2.82×10^{15}

[a] CAD, collisionally activated dissociation.

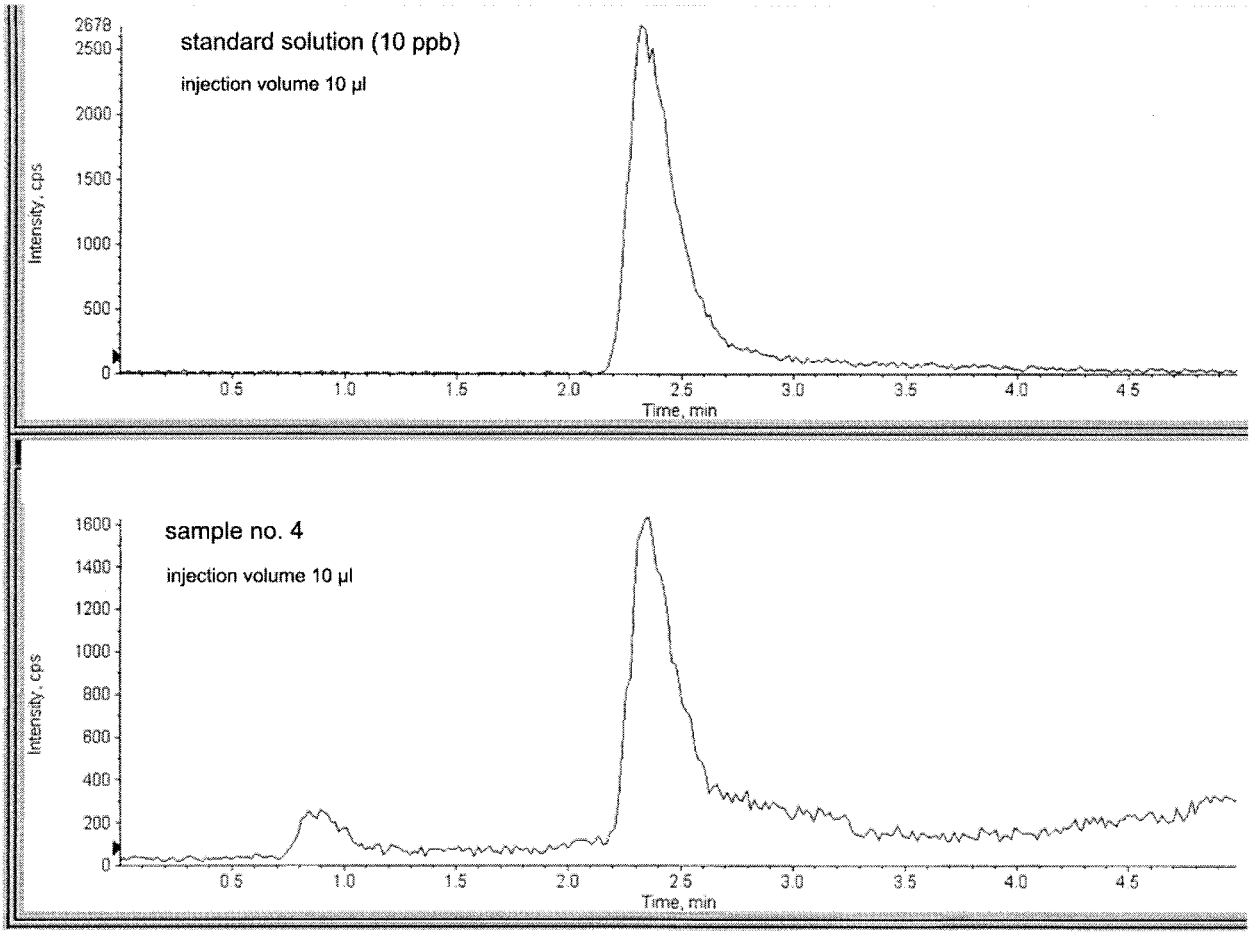

FIG. 1. Chromatogram of sample 4 and of sterigmatocystin standard solution (10 ppb). The injection volume was 10 µl.

matocystin/g of dust (Table 3). In both samples, the concentrations of culturable *A. versicolor* were $>10^5$ CFU/g of dust. However, other samples with even higher concentrations of *A. versicolor* did not show detectable sterigmatocystin. The two sterigmatocystin-positive samples were collected in July 1999 and March 2000. The dwellings evidenced no obvious differences from those without detectable sterigmatocystin.

TABLE 3. Culturable fungi, *A. versicolor*, sterigmatocystin, and toxigenic *A. versicolor* isolates in 11 carpet dust samples from eight dwellings with visible mold growth and/or signs of indoor dampness[a]

Sample no.	Amt (CFU/g of dust) of:		% of *A. versicolor* or culturable fungi	Amt of sterigmatocystin (ng/g of dust)[b]	No. of toxigenic *A. versicolor* isolates/ total no. investigated[c]
	Culturable fungi	*A. versicolor*			
1	8.2×10^4	1.7×10^3	2.0	<DL[d]	2/2
2	1.4×10^6	2.9×10^5	21.0	3.8/2.0[e]	18/18
3a	1.3×10^3	2.8×10^2	21.7	<DL	5/6
3b	1.0×10^3	0.8×10^2	8.4	<DL	1/1
4	3.2×10^6	1.7×10^5	5.2	3.4/3.8[e]	4/4
5	2.9×10^6	2.1×10^5	7.2	<DL	5/5
6a	4.5×10^5	3.6×10^5	80.0	<DL	4/4
6b	7.4×10^4	1.3×10^4	17.6	<DL	2/2
6c	9.0×10^4	3.1×10^4	34.4	<DL	3/3
7	8.7×10^5	5.3×10^5	60.9	<DL	5/5
8	1.4×10^5	$<2.5 \times 10^1$	<0.1	<DL	0/0

[a] *A. versicolor* results both absolute and relative to culturable fungi are presented.
[b] HPLC data.
[c] Number of toxigenic *A. versicolor* isolates/total number of isolates investigated by TLC.
[d] DL, detection limit (1.0 ng/g).
[e] Results of duplicate extractions.

DISCUSSION

Our pilot study confirms that *A. versicolor* is a common indoor fungus in damp houses, at least in northern Germany, accounting for a median proportion of culturable fungi in carpet dust of 18%. Hodgson and Scott (19) reported that *A. versicolor*, when present as the major or dominant taxa (i.e., accounting for >20% of fungal concentration), is generally associated with problem buildings and thus can be used as an indicator of fungal contamination. In view of the problems arising with air sampling (notably, temporal variability in levels of airborne fungal spores, time needed, and expense of sampling), dust sampling of viable fungi might be considered for large-scale epidemiological studies to estimate exposure to fungi (25, 43).

Our investigation shows that nearly all strains of *A. versicolor* isolated from carpet dust were able to produce sterigmatocystin in vitro. In published data, the proportion of in vitro sterigmatocystin-producing isolates from different sources (ham, spices, roughage, indoor air, moldy building materials, and composting plants) varies from 60 to 100% (12, 15, 21, 29, 31). Moreover, our investigation shows that sterigmatocystin may be detected in native carpet dust samples from damp indoor environments. To our knowledge, this is the first report concerning the detection of sterigmatocystin in this matrix. It is not clear, however, if the carpet was the location in which sterigmatocystin was actually produced, or simply represented a type of passive sampler. Systematic data on the degradation of sterigmatocystin in carpet dust over time and on the environmental conditions favoring production of sterigmatocystin on building materials and furnishings are widely lacking; additional research is needed in this area.

During an outbreak of building-associated pulmonary disease in a courthouse in Florida, *A. versicolor* was reported to be the dominant taxa in indoor air samples; however, quantification of sterigmatocystin was not possible "because of interfering peaks on HPLC chromatograms, even after extensive clean-up" (20). The analytical method used in our study was highly specific to the determination of sterigmatocystin. Identification by MS/MS and quantification by ESI have also been applied for the determination of other mycotoxins: e.g., aflatoxins (26), ochratoxin A (23, 28), fumonisin B1 (24), and polar and macrocyclic trichothecene mycotoxins (41). The poor recovery from our extraction and purification procedure was likely due to physical-chemical interactions of sterigmatocystin with the enormous dust surface in combination with the very low spiking concentrations relative to those used in other studies (39). However, this does not disqualify the basic findings of our pilot study. In the study by Tuomi and coworkers (42), the corresponding yields ranged from 7 to 92% for different mycotoxins in bulk samples of moldy interior finishes, with a median yield of <50%.

Apart from direct skin contact with moldy materials, indoor exposure to sterigmatocystin may occur by ingestion of house dust and by inhalation of aerosolized particles. For toddlers and preschool children, the target group of most concern, an oral uptake of 20 to 100 mg of house dust and an inhalative uptake of 0.5 mg of aerosolized dust per day are assumed as standard levels in Germany (38). In our investigation, both the proportion of sterigmatocystin-positive carpet dust samples from damp homes and the concentrations of sterigmatocystin in carpet dust were low, the latter being in the range of <10 ng/g of dust. Given these determinants, the daily uptake (by toddlers and preschool children) of sterigmatocystin by ingestion and inhalation of dust can thus be estimated to be <1 ng in those dwellings. This estimation is limited by the fact that dust is a difficult matrix for chemical analyses, and any assessment of environmental exposures based solely on vacuum cleaner sampling is prone to a high level of errors and misjudgment (40).

Conclusions. From this preliminary study, we conclude that most strains of *A. versicolor* isolated from carpet dust are able to produce sterigmatocystin in vitro and that sterigmatocystin may occasionally occur in carpet dust from damp indoor environments. Further research and systematic field investigation are needed to confirm our results and to provide an understanding of the health implications of mycotoxins in indoor environments.

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